Grape Berry Transpiration Is Determined by Vapor Pressure Deficit, Cuticular Conductance, and Berry Size

Yun Zhang[1]* and Markus Keller[2]

Abstract: Developmental changes and factors determining grape berry transpiration were investigated in three genetically diverse *Vitis* cultivars. Transpiration rates were measured on whole clusters, using a custom-designed cluster chamber, and on individual berries by weighing detached berries over time. Results obtained using the two methods were in good agreement. The chamber method verified the assumption that detaching berries does not alter their transpiration and also showed that rachis transpiration was minor compared to whole cluster transpiration. Berry transpiration fluctuated with vapor pressure deficit which was the main determinant of the driving force. The transpiration rate per berry and, to a lesser extent, the cuticular conductance, peaked when berries were red/purple (~13 Brix) and then declined with further ripening. Due to the decline in cuticular conductance during late ripening, the positive linear relationship between berry transpiration rate and surface area weakened after berries ripened. Despite the similar developmental patterns of berry transpiration and cuticular conductance, Concord (*V. labruscana*) berries consistently had much lower cuticular conductance than Merlot and Syrah (*V. vinifera*) berries. These results showed that berry transpiration was determined by both external factors (air temperature and relative humidity) and cultivar-specific internal factors (primarily berry surface area and cuticular conductance).

Key words: cuticular conductance, fruit transpiration, grape berry, ripening, *Vitis*

Water comprises from 70% to >90% of the fresh weight of grape berries at maturity and also determines the concentrations of solutes such as sugars, organic acids, minerals, and phenolic compounds (Keller 2015). In grape berries, as in other fleshy fruits, transpiration contributes to fruit weight loss (Rogiers et al. 2004, Clearwater et al. 2012) and affects fruit ripening and solute accumulation (Rebucci et al. 1997, Morandi et al. 2010). Therefore, understanding how developmental changes and other factors influence berry transpiration is important to improve practices to manipulate yield and quality of grapes.

Fruit transpiration is often measured by weighing detached fruits over time (Lang 1990, Rogiers et al. 2004, Morandi et al. 2010). Based on the assumption that detaching does not alter fruit transpiration (Lang 1990), the transpiration rate (E) is calculated as the rate of weight loss over time. However, whether or not cutting off the water supply to the fruit alters its transpiration has not been tested. Poni et al. (2001) challenged this assumption and used a custom-built system to measure whole cluster transpiration in situ, but did not compare their results with the weighing method to validate it. Berry E is usually much lower than that of leaves (Rogiers et al. 2004, Keller 2015), indicating a very low surface conductance (g) to water vapor. The number of stomata on the berry surface is less than one per mm^2 (Blanke and Leyhe 1988), and the stomata become partially or completely occluded by epicuticular wax or are transformed into lenticels after fruit set (Blanke and Lenz 1989, Hardie et al. 1996). Therefore, berry transpiration is predominantly cuticular and lacks stomatal regulation (Possingham et al. 1967). As a result, E per unit surface area (A) is strongly affected by environmental factors such as ambient temperature (T) and relative humidity (RH), which determine the vapor pressure deficit (VPD; Gibert et al. 2005, Nobel 2009).

E declines more than five-fold from unripe to ripe berries, both on a per surface area basis and on a per berry basis (Rogiers et al. 2004). A developmental decrease in E has also been reported for kiwifruit berries (Montanaro et al. 2012), and has been attributed to anatomical modifications of the fruit epidermis (Hallett and Sutherland 2005). This indicates that g may change during development. Due to the lack of functional stomata (Blanke and Leyhe 1988), grape berry g consists of the boundary layer conductance (g_b) and cuticular conductance (g_c). Because g_c is one or two orders of magnitude lower than g_b (Nobel 2009), g_c exerts the main limitation on berry E and thus $g \approx g_c$ (Gibert et al. 2005). Estimates of g or g_c have been reported for sweet cherries (Knoche et al. 2000), kiwifruit berries (Clearwater et al. 2012), and tomato fruit (Leide et al. 2007). However, little information is available for grape berry g_c.

To better understand developmental changes and other factors determining berry transpiration, we studied berries

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of three genetically distinct grape cultivars. We first tested the assumption that detaching berries does not alter berry E, which was measured using two different methods and cross-validated. Also, the relationship between VPD and E was studied. Finally, developmental changes and cultivar differences in berry E and g_c were investigated.

Materials and Methods

Plant material. Own-rooted grapevines, *Vitis vinifera* L. cvs. Merlot and Syrah (planted in 1999) and *V. labruscana* Bailey cv. Concord (planted in 2003) were used for field measurements and sample collection in 2011 and 2012. These vines were grown with north-south row orientation and 1.8 m × 2.7 m spacing in the experimental vineyard of the Irrigated Agriculture Research and Extension Center in Prosser, WA (lat. 46°17'N; long. 119°44'W; elevation 365 m asl). Mean annual and growing season (April through October) precipitations were 171 mm and 92 mm, respectively, over the study years. All vines were drip-irrigated. Merlot and Syrah were trained to a bilateral permanent cordon with spur pruning and loose vertical shoot-positioning, and Concord was mechanically pruned with no shoot-positioning. No shoot thinning or other canopy management practices were applied during this study.

Two-year-old, own-rooted Syrah and Concord grapevines were grown in white PVC pots containing a mixture of 50% sandy loam, 25% peat moss, and 25% pumice with an addition of 30 g/L dolomite. These vines were grown in an air-conditioned greenhouse (temperature 18 to 30°C; midday photosynthetically active radiation >1000 µmol photons/m^2 sec). All pots were irrigated regularly to prevent water stress.

Grape berries are often classified as preveraison, veraison, or postveraison. However, during veraison the berries undergo many physiological processes that do not occur simultaneously or over the same time scale (Coombe 1992), and berry skin color can vary from green to blue on a single cluster. Consequently, berries were grouped into successive developmental stages based on their firmness to the touch and on skin color (Keller and Shrestha 2014, Keller et al. 2015), and were coded as follows: green hard = 1, green soft = 2, blush/pink = 3, red/purple = 4, or blue = 5. After the berries turned blue, two more groups were determined according to total soluble solids (TSS): ripe = 6 (Merlot and Syrah: 20 to 24 Brix; Concord: 18 to 20 Brix) and overripe = 7 (Merlot and Syrah: >24 Brix; Concord: >20 Brix). Strong correlations were obtained between juice solute concentration, measured as TSS using a hand-held refractometer (Atago, Tokyo, Japan), and developmental stages (codes) for each cultivar ($r > 0.96$, $p < 0.001$, n = 50).

Model of grape berry transpiration. Symbols, units of measurement, and parameters used in the grape berry transpiration model are listed in Table 1. According to Fick's law (Nobel 2009), the rate of berry transpiration (E) can be described as

$$E = g_c \times \Delta N = g_c \times (\Delta P/P^*) \qquad \text{Eq. 1}$$

where $\Delta N = N_i - N_a$ and $\Delta P = P_i - P_a$; N_i and P_i are the mole fraction and partial pressure, respectively, of water vapor in the internal air space (or apoplast) of the berry; N_a and P_a are the mole fraction and partial pressure, respectively, of water vapor in the ambient air; and P^* is the standard atmospheric pressure. From Equation 1, g_c can be calculated as

$$g_c = E/\Delta N = E \times P^*/\Delta P = E \times P^*/(P_i - P_a) \qquad \text{Eq. 2}$$

It is often assumed that the air space inside a plant organ is saturated with pure water, so that P_i equals the partial water vapor pressure of saturated air (P_s; Nobel 2009, Montanaro et al. 2012). However, solutes accumulate in the apoplast of ripening berries (Wada et al. 2009, Keller and Shrestha 2014), which lowers P_i according to Raoult's law (Nobel 2009). Therefore, P_i was estimated using the water potential of the berry apoplast (Ψ_i). As a simplification, we assumed that $\Psi_i \approx \Psi_\pi$ (the osmotic potential of berry apoplast), and Ψ_π was assumed to be equal to the berry osmotic potential (= 0.31 – 0.20 × TSS; Bondada and Keller 2012). Based on the definitions of RH and water potential of water vapor (Nobel 2009),

$$P_i = RH_i \times P_s = e^{\frac{\Psi_i \times \overline{V}_w}{R \times T}} \times P_s \qquad \text{Eq. 3}$$

where RH_i is the relative humidity inside the berry, R is the gas constant, T is the thermodynamic temperature, and $\overline{V}_w$

Table 1 Symbols, units of measurement, and parameters used in the text and the equations of the fruit transpiration model.

Symbol	Definition	Unit
A	Grape berry surface area	m^2
E	Grape berry transpiration rate	µmol H_2O/m^2 sec
E_b	Daily E on a per berry basis	mg H_2O/day
E_b/A	Daily E on a per surface area basis	g H_2O/m^2 day
E_b/A'	Calculated E_b/A for cross-validation	g H_2O/m^2 day
E_c	Whole cluster E	µmol H_2O/sec
E_c^b	Berry E calculated using E_c, the number of berries in the cluster, and berry surface area	µmol H_2O/m^2 sec
$E_c^{b'}$	Calculated E_c^b for cross-validation	µmol H_2O/m^2 sec
g	Surface conductance to water vapor	mmol H_2O/m^2 sec
g_b	Boundary layer conductance	mmol H_2O/m^2 sec
g_c	Cuticular conductance	mmol H_2O/m^2 sec
N	Mole fraction of water vapor	dimensionless
N_i	N in the internal air space of the berry	dimensionless
N_a	N in the ambient air	dimensionless
P	Partial pressure of water vapor	kPa
P_i	P in the internal air space of the berry	kPa
P_a	P in the ambient air	kPa
P_s	P in the saturated air	kPa
P*	Standard air pressure (= 101.325)	kPa
RH	Relative humidity (RH; between 0 and 1)	dimensionless
RH_i	RH in the internal air space of the berry	dimensionless
RH_a	RH in the ambient air	dimensionless
Ψ_i	Water potential of the berry apoplast	MPa
Ψ_π	Osmotic potential of the berry apoplast	MPa
T	Temperature	°K or °C
R	Gas constant (= 8.3144621)	cm^3 MPa/°K mol
$\overline{V}_w$	Partial molar volume of liquid water (= 18)	cm^3/mol

is the partial molar volume of liquid water. Combining Equations 2 and 3,

$$\Delta P = e^{\frac{\Psi_i \times \overline{V}_w}{R \times T}} \times P_s - RH_a \times P_s = (e^{\frac{\Psi_i \times \overline{V}_w}{R \times T}} - RH_a) \times P_s \quad \text{Eq. 4}$$

where RH_a is the relative humidity of ambient air.

For comparison, g_c was also estimated based on the aforementioned common assumption that $P_i = P_s$ (that is $\Delta P = VPD$).

Measurement of cluster transpiration using a cluster chamber. A fruit cluster chamber constructed from two halves of a hollow cylinder made of clear acrylic plastic (245 mm in high, 125 mm in diam, and 3 mm wall thickness) was custom-designed to measure whole-cluster transpiration in situ. The chamber opened by pivoting along one side of the two halves, which were connected by screws. A hinge was used on the other side to close the chamber. A 10 mm × 10 mm hole was cut through the top of the chamber. To measure transpiration, a cluster was enclosed in the chamber with its peduncle protruding through the hole. Foam insulation was glued around the hole and along the sides of the half-cylinder to prevent air leakage when the chamber was closed. A temperature sensor and two RH sensors (ADC BioScientific, Herts, UK) were mounted inside the chamber to monitor T and reference and analysis RH. During a preliminary experiment, both chamber and ambient T were tracked using two temperature sensors (Thermochron iButton; Maxim Integrated, San Jose, CA). Over an ambient T range of 22 to 56°C, the chamber T was consistently 1.86°C higher than the ambient T with no sign of overheating inside the chamber (Chamber T = 0.98 × Ambient T + 1.86, $r = 0.98$, $p < 0.001$; see also Supplemental Figure 1). A light sensor (ADC BioScientific) was mounted on and perpendicular to the top of the chamber. The chamber was connected with the console of an LC*pro*+ portable photosynthesis system (ADC BioScientific) through its handle. The rate of air flow to the chamber was 335 ± 5 µmol/sec. Cluster transpiration rate (E_c) was calculated based on the difference in reference and analysis RH and the air flow rate.

To test the assumption that detachment does not alter transpiration, E_c was measured in situ on field-grown Syrah vines continuously for 1 hr. Then the clusters were detached from the vines, kept at the same location in the canopy, and E_c was recorded for another 1 hr. Measurements were recorded automatically every 2 min. Sixteen clusters were tested during different two-hour windows over several days. The E_c of each cluster before and after detaching and the corresponding VPD (calculated from T and RH; Nobel 2009) were compared using a paired *t*-test (Statistica 7.0; StatSoft, Inc., Tulsa, OK).

In a separate experiment, the contribution of the rachis to E_c was tested. The E_c of 10 clusters of pot-grown Syrah vines was measured using the cluster chamber for 1 hr each in the greenhouse. Then, the rachis (but not the pedicels and receptacles) of each cluster was carefully covered with antitranspirant (2% v/v di-1-*p*-menthene; Vapor-Gard, Miller, Hanover, PA) using a small paintbrush, and E_c was measured again for 1 hr. A preliminary experiment showed that berry transpiration was reduced by 30% with Vapor-Gard applied on the berry surface (Y. Zhang and M. Keller, unpublished results), which was somewhat less than the previously reported 50% reduction in leaf transpiration (Palliotti et al. 2013). The E_c of each cluster with and without antitranspirant and the corresponding VPD during the measurements were compared using a paired *t*-test.

E_c of clusters of pot-grown Concord and Syrah vines was measured in the greenhouse under varying VPD. For each cultivar, three clusters were measured at each of three stages: preveraison (all berries were green hard), veraison (berries ranging from blush/pink to red/purple), and postveraison (all berries had turned blue). In addition, three preveraison Syrah clusters were measured outside the greenhouse during a heat wave (maximum ambient T ~45°C). The reason that these stages were used to categorize clusters was the asynchronous development of berries on one cluster during veraison (Coombe 1992). The transpiration rate per berry measured using the cluster chamber (E_c^b) was calculated by dividing E_c by the number of berries on each cluster. Relationships between E_c^b and VPD were evaluated by correlation analysis (Statistica 7.0).

Berry transpiration measurements by weighing detached berries over time. Berries of Concord, Merlot, and Syrah were randomly sampled in the experimental vineyard from green hard (preveraison) to overripe. They were immediately transferred to the laboratory in a sealed plastic bag inside a cooler. Berries were grouped into developmental stages as before. For each stage, five or ten 10-berry replicates were used to estimate berry transpiration under constant VPD (1.35 kPa) as described in Keller et al. (2015). Berry TSS of an additional five 10-berry replicates was measured at 0 hr. Berry volume was estimated from berry weight and density calculated from tabulated values of TSS and density (density (kg/L) = 0.9984 + 0.0038 × TSS + 0.00001631 × TSS^2). Berry A was calculated from berry volume, based on the properties of a sphere. Daily transpiration was expressed both on a per berry basis (E_b) and on a per surface area basis (E_b/A). Next, g_c of each cultivar and developmental stage was calculated using Equation 2. The variation in A, E_b, E_b/A, and g_c due to cultivar and developmental stage was evaluated by two-way ANOVA (Statistica 7.0). Due to significant interaction among cultivars and developmental stages, one-way ANOVA was then conducted and differences due to developmental stages within each cultivar were evaluated using Fisher's least significant difference test. The relationship between E_b and A was evaluated by correlation analysis.

An additional set of 100 berries of each cultivar at each of two developmental stages, green hard and blue, were collected intentionally to cover a large range of berry sizes. Their individual E_b and A were estimated as described above, and the relationship between E_b and A was analyzed by correlation analysis.

Cross-validation of berry transpiration rates measured using two methods. To cross-validate the two methods used to measure berry transpiration, the values obtained using the cluster chamber and by weighing detached berries were compared. First, berry transpiration under the conditions experienced during the chamber measurements was calculated ($E_c^{b'}$)

using Equation 1. For this purpose, g_c was obtained using the weighing method, and ΔP was calculated from T and RH recorded by the cluster chamber using Equation 4. Then, the relationship between E_c^b' and E_c^b was evaluated using correlation analysis. Second, daily berry transpiration rate per surface area under laboratory conditions was calculated (E_b/A') using the regression equations between E_c^b and VPD obtained from the cluster chamber experiments. Then E_b/A' was compared with E_b/A at three stages (preveraison, veraison, and postveraison). No statistical analysis could be conducted for this comparison because the calculated E_b/A' could not be replicated. Therefore, the comparison between E_b/A' and E_b/A was only to test whether the calculated values were in the same range as the measured ones.

Results

Detaching the clusters from field-grown vines did not change E_c significantly when compared with E_c measured before detaching (32 ± 11 and 35 ± 11 μmol H_2O/sec, before and after detaching, respectively, n = 16, $p = 0.54$). No difference in E_c was found before and after the rachis of pot-grown vines was covered with antitranspirant (5.3 ± 1.3 and 5.4 ± 1.2 μmol H_2O/sec, with or without antitranspirant, respectively, n = 10, $p = 0.45$). Therefore, cluster transpiration was dominated by berry transpiration. Due to differences in VPD (2.7 kPa in the field and 1.9 kPa in the greenhouse) and cluster size (≥150 berries per cluster on field-grown vines but only 30 to 90 berries per cluster on pot-grown vines), the absolute values of E_c between these two experiments were rather different. Nevertheless, this difference was irrelevant to the abovementioned conclusion.

Diurnal changes in E_c^b closely followed fluctuations in VPD (Figure 1). Daily berry E varied between 14 and 20 mg H_2O/day during this experiment. Furthermore, E_c^b exhibited a close linear relationship with VPD from preveraison to postveraison (Figure 2), even when VPD went up to 8 kPa during a heat wave (inset in Figure 2A). Under the same VPD, E_c^b was slightly higher at veraison than at either pre- or postveraison (Figure 2), which implies a developmental change in berry g_c.

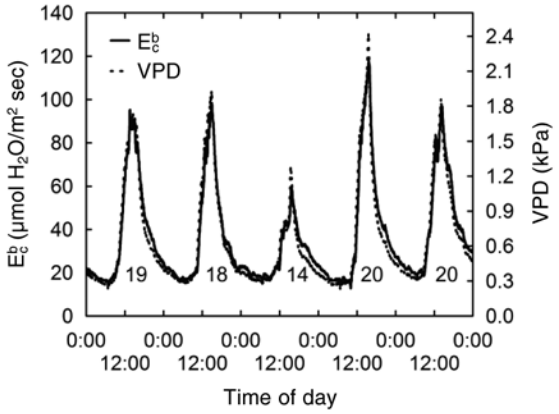

Figure 1 Diurnal changes in grape berry transpiration rate (E_c^b) and vapor pressure deficit (VPD) measured over five days on a preveraison cluster of a pot-grown Syrah vine using a cluster chamber. Numbers underneath the peaks indicate the integrated daily berry transpiration (mg H_2O/day).

Average daily E_b for all three cultivars, measured by weighing detached berries, was lowest at the green hard stage (20.8 to 24.9 mg H_2O/day) and peaked at the red/purple stage ($p < 0.001$), constituting a 1.7-, 1.7-, and 2.4-fold increase for Concord, Merlot, and Syrah, respectively (Figure 3A). E_b declined with further ripening ($p < 0.001$; 1.2-, 1.5-, and 1.6-fold decrease for Concord, Merlot, and Syrah, respectively; Figure 3A). Although Concord berries were much larger than Merlot and Syrah berries (Figure 3C), the E_b of Concord was either less than or in between that of Merlot and Syrah. Developmental changes in E_b/A exhibited a similar pattern as E_b, peaking at the red/purple stage for these three cultivars ($p < 0.001$; Figure 3B). No difference in E_b/A was found between Merlot and Syrah ($p = 0.92$), except at the ripe and overripe stages ($p < 0.001$). However, the average E_b/A of Concord was 40% lower than that of Merlot and Syrah ($p < 0.001$). Since the measurements were taken in the same environment for all cultivars, the differences in E_b/A imply genetic differences in berry g_c.

When measured under identical conditions, E_b increased linearly as berry A increased from the green hard to the blue

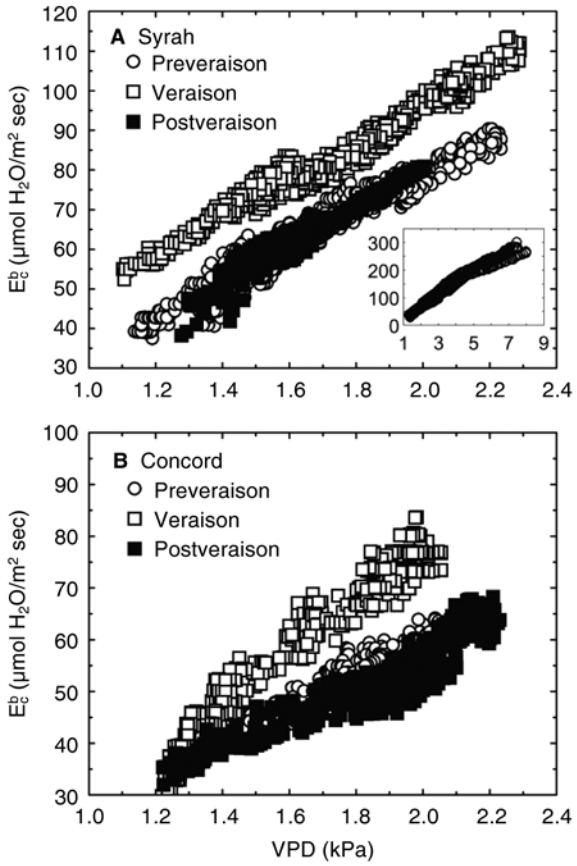

Figure 2 Correlation between vapor pressure deficit (VPD) and grape berry transpiration rate (E_c^b) measured on potted vines at preveraison (all berries were green hard), veraison (berries varied from blush/pink to red/purple), and postveraison (all berries were blue) using a cluster chamber. (**A**) Syrah: preveraison: y = 40x - 3; veraison: y = 47x + 1; postveraison: y = 41x - 6; (**B**) Concord: preveraison: y = 25x - 3; veraison: y = 38x - 10; postveraison: y = 29x - 6. All $r \geq 0.97$, $p < 0.001$, n ≥ 650. Inset in (**A**) shows a similar relationship between VPD and (E_c^b) of preveraison Syrah clusters at much higher VPD (y = 41x -11, $r = 0.99$, $p < 0.001$, n = 564).

stage (TSS ≤ 20 Brix; Figure 4A). However, the relationship between A and E_b became weaker when berries with TSS >20 Brix (ripe and overripe stages) were included in the analysis (Figure 4B). This implies that, irrespective of cultivar, a decrease in g_c was responsible for the lower E_b at advanced ripening stages. The relationship between E_b and A was further tested in 100 additional berries of each cultivar at the green hard and blue stages. Again, E_b exhibited positive linear relationships with A, which ranged from 225 to 584 mm^2 for green hard berries, and from 296 to 1059 mm^2 for blue berries (Figure 5). These linear relationships under the same VPD at a specific developmental stage also implied constant g_c at that stage. At both stages, the slopes of the linear regressions for Merlot and Syrah were similar, while for Concord, E_b increased less with increasing A, again indicating lower berry g_c for Concord than Merlot and Syrah.

Consistent with this notion, g_c calculated using Equation 2 was much higher overall for Merlot and Syrah than for Concord ($p < 0.001$; Table 2). For Concord berries, g_c was highest at the red/purple and blue stages ($p < 0.001$). After the berries turned ripe, g_c declined to its lowest level ($p < 0.001$). Similar to Concord, g_c of Merlot and Syrah berries was highest at the red/purple stage ($p < 0.001$). Different from Concord, g_c started decreasing as Merlot and Syrah berries progressed from red/purple to blue ($p < 0.001$). Although Ψ_i decreased from -0.8 MPa to -4.8 MPa during ripening, this only caused a slight decrease in RH_i (from 0.99 to 0.97) and ΔP (from 1.28 kPa to 1.22 kPa). Consequently, under the conditions of this study, the assumption $P_i = P_s$ led to an overestimation of P_i by 0.7 (preveraison) to 3.5% (overripe) and an underestimation of g_c by 1.4 to 7.3%.

The results for berry transpiration obtained using two different methods, the cluster chamber and weighing detached berries, were cross-validated. The E_c^b' (calculated using g_c from the weighing method) had a close linear relationship with E_c^b measured for both Syrah and Concord (Figure 6). The relationship for Syrah was close to 1:1, but for Concord, the calculated E_c^b' underestimated the measured E_c^b. The E_b/A'

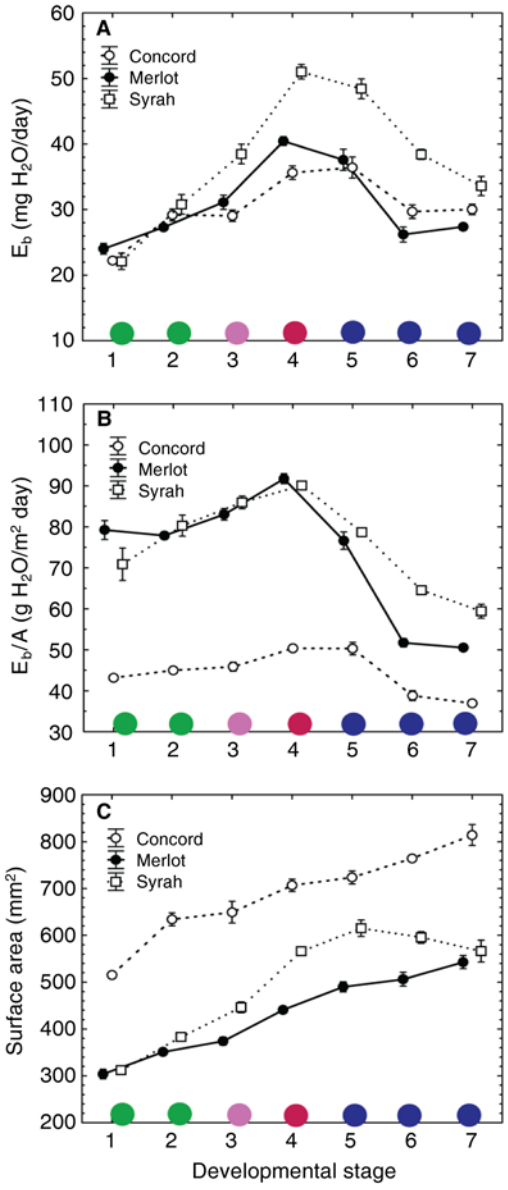

Figure 3 Grape berry (**A**) transpiration rate per berry (E_b), (**B**) transpiration rate per surface area (E_b/A), and (**C**) surface area of Concord, Merlot, and Syrah at successive developmental stages (1 = green hard; 2 = green soft; 3 = blush/pink; 4 = red/purple; 5 = blue; 6 = ripe; 7 = overripe). The color of the circle above the x-axis indicates the color of the berry skin at each stage. Transpiration was measured by weighing detached berries over 48 hr in a controlled environment. Data are means ± SE where SE > symbol size (n ≥ 5, 10 berries per replicate).

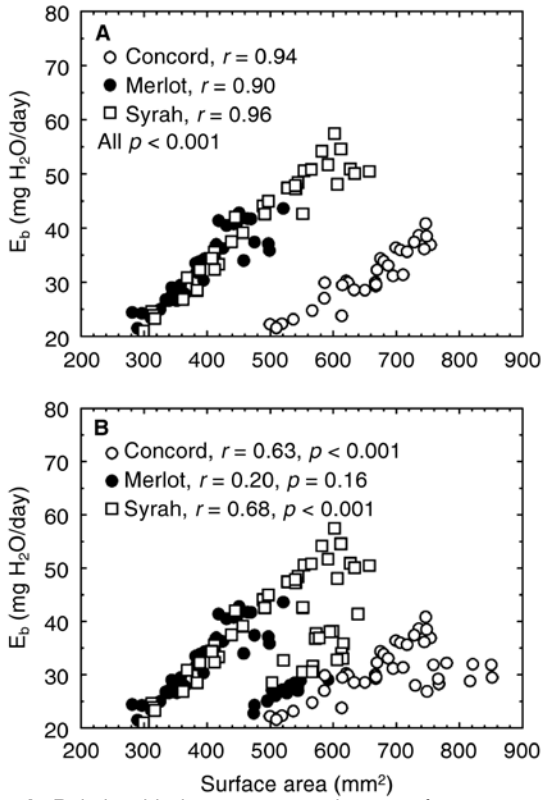

Figure 4 Relationship between grape berry surface area and berry transpiration rate (E_b). (**A**) Berries from green hard to blue stages (total soluble solids (TSS) ≤ 20 Brix, n = 25 per cultivar). (**B**) Ripe and overripe berries (20 Brix ≤ TSS ≤ 27 Brix) added to the berries presented in (**A**) (n = 35 per cultivar). Transpiration was measured by weighing detached berries over 48 hr in a controlled environment.

calculated using the regression equations obtained from the chamber measurements (see Figure 2) was in the same range as E_b/A measured in the weighing experiments (Table 3). The largest variation was at veraison (12.2 and 16.7 g H_2O/m^2 day for Syrah and Concord, respectively), which was probably due to the asynchrony of berry ripening within one cluster at veraison.

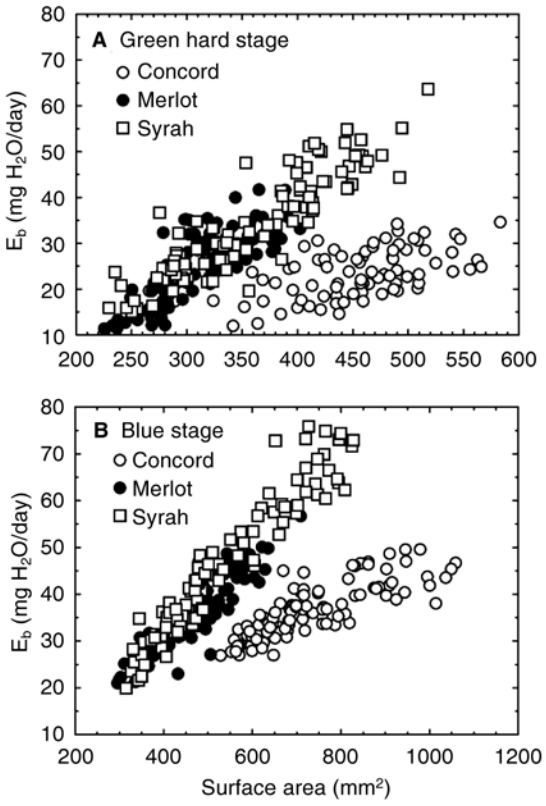

Figure 5 Correlation between grape berry surface area and berry transpiration rate (E_b) of Concord, Merlot, and Syrah at green hard (**A**) and blue (**B**) stages. (**A**) Concord: y = 0.05x + 2, r = 0.61; Merlot: y = 0.15x - 20, r = 0.80; Syrah: y = 0.15x - 18, r = 0.89. (**B**) Concord: y = 0.04x + 10, r = 0.81; Merlot: y = 0.08x - 2, r = 0.92; Syrah: y = 0.10x - 7, r = 0.96. All p < 0.001, n = 100. Transpiration was measured by weighing detached berries over 48 hr in a controlled environment.

Discussion

The presented results demonstrate that grape berry transpiration is mainly driven by VPD (Figures 1 and 2), depends on berry surface area (that is berry size; Figures 4 and 5), and is modulated by g_c (Table 2). Although berries

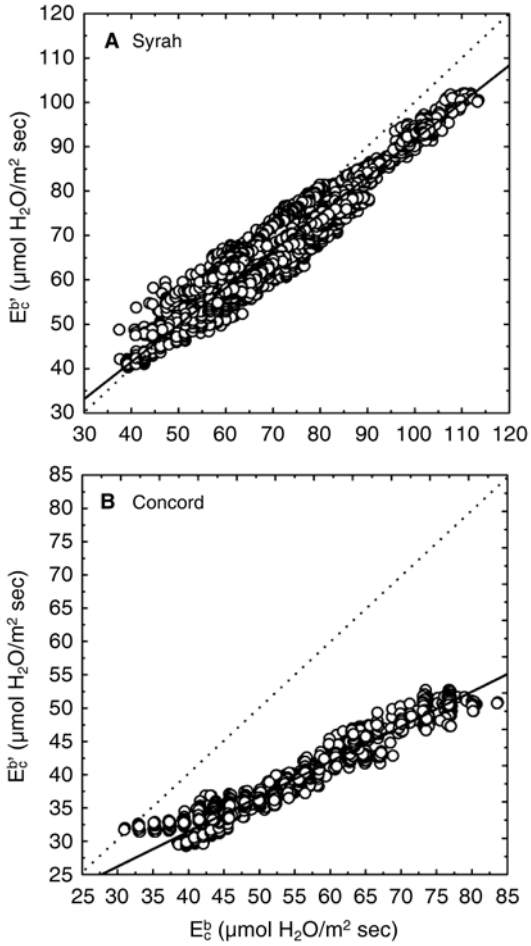

Figure 6 Correlation between grape berry transpiration rates measured (E_c^b) using a cluster chamber from preveraison to postveraison and estimated ($E_c^{b'}$) using temperature and relative humidity recorded by the chamber and cuticular conductance. Dotted line indicates 1:1 ratio. (**A**) Syrah: y = 8 + 0.83x; (**B**) Concord: y = 10 + 0.52x. For both, $r \geq 0.96$, p < 0.001, and n > 1500.

Table 2 Cuticular conductance (g_c) of Concord, Merlot, and Syrah grape berries at varying developmental stages. The g_c was calculated as the rate of berry transpiration per surface area divided by the gradient of the mole fraction of water vapor between the ambient air and the berry apoplast (Equation 2). Data are presented as means ± SE (n = 5; 10 berries per replicate). Different upper case letters indicate significant difference among genotypes within each stage; different lower case letters indicate significant difference among stages within each genotype (p < 0.05, Fisher's least significant difference test).

			g_c (mmol H_2O/m^2 sec)[a]		
Stage	**Code**	**Phenology**	**Concord**	**Merlot**	**Syrah**
Green hard	1	Preveraison	2.2 ± 0.02 C cd	4.0 ± 0.12 A c	3.6 ± 0.20 B d
Green soft	2	Veraison	2.3 ± 0.02 B bc	4.0 ± 0.05 A c	4.1 ± 0.13 A c
Blush/pink	3	Veraison	2.4 ± 0.08 B b	4.3 ± 0.07 A b	4.4 ± 0.10 A b
Red/purple	4	Veraison	2.6 ± 0.06 B a	4.7 ± 0.06 A a	4.7 ± 0.05 A a
Blue	5	Postveraison	2.6 ± 0.08 B a	4.0 ± 0.11 A c	4.1 ± 0.04 A c
Ripe	6	Postveraison	2.1 ± 0.07 C de	2.8 ± 0.05 B d	3.4 ± 0.02 A d
Overripe	7	Postveraison	2.0 ± 0.04 C e	2.7 ± 0.02 B d	3.2 ± 0.09 A e

[a]The unit mmol H_2O/m^2 sec may be converted to cm/hr by multiplying the presented values with 9.45.

transpire at much lower rates than do leaves (Keller et al. 2015), unlike leaves, ripening berries are unable to control their E via stomatal closure (Possingham et al. 1967, Blanke and Leyhe 1988). Therefore, berry E closely traces changes in VPD. Nevertheless, under the same VPD, berry E differs due to both berry size and g_c, both of which vary by cultivar and developmental stage. All three cultivars tested here shared the same developmental pattern of berry E and g_c (Figure 3A and B, Table 2). The transient increase in E_b observed at the beginning of ripening was mostly attributable to berry expansion during the second growth phase (37, 45, and 82% increase in berry surface area of Concord, Merlot, and Syrah, respectively; Figure 3C) and, to a lesser extent, an increase in g_c (18, 18, and 31% increase in Concord, Merlot, and Syrah, respectively; Table 2). During late ripening, the decline in g_c (Table 2) resulted in a decline in E_b despite increasing or stable berry surface area (Figures 3C and 4B). Thus, the positive linear relationship between E_b and A under the same VPD was sustained until the blue stage (Figure 4A), and then disappeared once the berries exceeded 20 Brix (ripe and overripe stages; Figure 4B). This suggests that, under constant VPD, the increase in berry A dominated changes in E_b during early ripening, but the decrease in g_c became dominant during late ripening. The three cultivars varied significantly in the absolute values of g_c (Table 2), which led to E_b/A in Merlot and Syrah being nearly twice that of Concord (Figure 3B).

Fruit transpiration rate is often estimated by measuring weight loss in detached fruits (Rogiers et al. 2004, Morandi et al. 2010, Becker and Knoche 2011). Our results support the assumption that detaching does not alter fruit transpiration, at least in the short term. Using the cluster chamber, no difference in E_c was found before and after detaching. Previously, we reported that weight loss from detached berries remained constant over three successive 24-hr periods (Keller et al. 2015). Also, rachis transpiration was insignificant for whole-cluster transpiration. This may be partly due to the small surface area of the rachis (~4%) relative to the whole cluster surface area (Poni et al. 2001). This conclusion is similar to the finding that the contribution of the pedicel/receptacle to overall berry transpiration was negligible (Becker and Knoche 2011). Furthermore, the rates of berry transpiration obtained using the cluster chamber and the weighing method agreed with each other (Table 3 and Figure 6).

The strong positive correlation between E_c^b and VPD (Figures 1 and 2) indicates that VPD (thus T and RH) is the dominant determinant of the driving force (ΔP) for berry transpiration. Unlike leaves, which may close their stomata to reduce transpiration, grape berries do not regulate their transpiration (Possingham et al. 1967), hence the linear relationship between E_c^b and VPD even up to 8 kPa (inset of Figure 2). This suggests that berries may be vulnerable to dehydration under high VPD. Although E_b was much lower than the leaf transpiration rate (2 to 14 mmol H_2O/m^2 sec; Keller 2015), it accounted for a daily loss of 2 to 5% of berry weight (23 to 54 mg H_2O/day; Figure 3A) in a controlled laboratory environment (VPD = 1.35 kPa). Daily mean VPD in the field during ripening may be higher (up to 2.7 kPa under the climatic conditions of this study; http://weather.wsu.edu). Based on the weather data during ripening of the two experimental years, E_b integrated over 24 hr varied between 3 and 100 mg H_2O/day. This is equivalent to 0.2 to 6% of berry fresh weight and is largely due to daily variation in VPD.

Our method of categorizing and measuring berries according to their firmness to the touch and skin color permitted insight into the details of the developmental changes in E during veraison. Using this sampling approach, we observed a two-phase pattern of developmental change in berry E that was consistent among the three cultivars (Figure 3A). This contradicts the previous finding of a steady decline in berry E from preveraison to postveraison (Rogiers et al. 2004). These authors may have missed the possible changes during veraison (e.g., TSS changed from 8 to 20 Brix from the green soft to the blue stage; Supplemental Figure 2) because no sample was taken during veraison (see Table 1 in Rogiers et al. 2004). Close examination of Figure 3B in Becker and Knoche (2011) also suggests that there might be a slight increase in berry E from 60 to 70 days after full bloom in Riesling.

Overall, grape berry g_c (2 to 4 mmol H_2O/m^2 sec; Table 2) is comparable to the g_c of grape leaves (~5 mmol H_2O/m^2 sec; Boyer et al. 1997), kiwifruit berries (~4 mmol H_2O/m^2 sec; Clearwater et al. 2012), and tomato fruit (0.4 to 6 mmol H_2O/m^2 sec; Leide et al. 2007). Yet the developmental pattern of grape berry g_c differed from that of kiwifruit berries and tomato fruit (Leide et al. 2007, Montanaro et al. 2012). Instead of declining during development, grape berry g_c of three cultivars consistently increased from the green hard to the red/purple stage, then decreased with further ripening (Table 2). This developmental pattern in g_c could explain why, on a per surface area basis, berry E at the same VPD was higher during veraison than preveraison or postveraison

Table 3 Comparison of two methods to estimate grape berry transpiration. Weighing method: transpiration per surface area was measured by weighing detached berries (E_b/A, means ± SE) at the green hard stage (preveraison); the blush/pink and red/purple stages (veraison); and the blue, ripe, and overripe stages (postveraison). Cluster chamber method: transpiration per surface area was calculated (E_b/A') using the regression equations for berry transpiration and vapor pressure deficit from the cluster chamber experiment at preveraison, veraison, and postveraison (see Figure 2).

	Syrah		Concord	
Stage	E_b/A (g H_2O/m^2 day)	E_b/A' (g H_2O/m^2 day)	E_b/A (g H_2O/m^2 day)	E_b/A' (g H_2O/m^2 day)
Preveraison	71.9 ± 4.0	79.3	43.2 ± 0.4	47.8
Veraison	88.0 ± 1.1	100.2	47.5 ± 0.7	64.2
Postveraison	67.5 ± 3.1	76.7	42.7 ± 2.7	51.6

(Figures 2 and 3B). Also, the decline in g_c during late ripening most likely contributed to the poor correlation between E_b and berry A (Figure 4B). Since the amount of cuticular wax per surface area does not increase during berry ripening (Grncarevic and Radler 1971, Rogiers et al. 2004), the decline in g_c after berries turned red/purple (Table 2) may be due to modifications in the chemical composition of the wax (Possingham et al. 1967, Riederer and Schreiber 2001, Leide et al. 2007). Comparing berry E with different fractions of the cuticular wax removed to that of control berries, Grncarevic and Radler (1971) concluded that soft wax, which consists of long-chain aliphatic compounds (e.g., alcohols, aldehydes, esters, free acids, and hydrocarbons; Radler 1965a), forms the main barrier to water evaporation. The significance of soft wax in fruit transpiration has also been demonstrated using the tomato mutant *lecer6* (Leide et al. 2007). This mutant is defective in a β-ketoacyl-coenzyme A synthase, an enzyme involved in very-long-chain fatty acid elongation (Vogg et al. 2004). The cuticle of *lecer6* fruit exhibited a decrease in very-long-chain alkanes and a distinct increase in water permeability compared to wild-type fruit (Leide et al. 2007). The amount of soft wax in grape berry cuticles increases from 40 days after full bloom (around veraison) to maturity (Yamamura and Naito 1983). This reported increase in soft wax coincides with the observed decline in E_b/A and g_c later during ripening (Figure 3B and Table 2). However, we currently do not have an explanation for the transient increase in g_c during early ripening (Table 2). In addition to these developmental changes, the amounts of soft and total wax on *V. labruscana* berries are more than twice those on *V. vinifera* berries (Grncarevic and Radler 1971, Yamamura and Naito 1983). Furthermore, the soft wax of *V. labruscana* berries may be much richer in hydrophobic hydrocarbons than that of *V. vinifera* berries (Radler 1965b). These findings are in agreement with the significantly lower E_b/A and g_c of Concord (*V. labruscana*) compared with Merlot or Syrah (*V. vinifera*) observed in this study (Figure 3B).

The relationships among internal and external factors that influence berry transpiration are presented in a flow chart (Figure 7). Berry surface area, Ψ_i, and g_c are internal factors under both genetic and environmental control: surface area increases with berry growth (Figure 3C), Ψ_i decreases with berry sugar accumulation, while g_c increases then decreases during berry ripening (Table 2). Because the effect of Ψ_i on ΔP is rather small, ambient T and RH (thus VPD) are the dominant factors determining the driving force (ΔP) for transpiration (Figures 1 and 2). Under constant VPD, E_b correlates positively with berry A (Figures 4A and 5; Becker and Knoche 2011); however, this relationship no longer holds once berries exceed 20 Brix due to the decline in g_c (Figure 4B and Table 2). Consequently, berry E changes diurnally due to fluctuations in VPD and developmentally due to berry growth and changes in g_c. Finally, despite a similar developmental pattern, cultivars differ in their berry E due to variations in berry size and g_c.

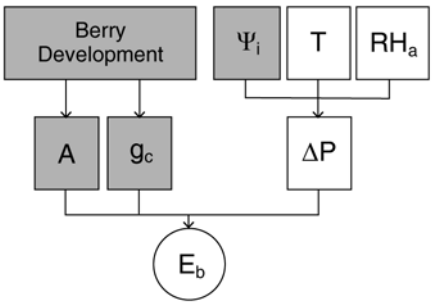

Figure 7 Internal (shaded rectangles) and external (open rectangles) factors that influence the rate of grape berry transpiration (E_b). Berry surface area (A), apoplast water potential (Ψ_i), and cuticular conductance (g_c) change during berry development. Ambient air temperature (T) and relative humidity (RH_a), i.e., vapor pressure deficit, are the main factors determining the driving force (ΔP) for berry transpiration, while the influence of Ψ_i is minor. Consequently, E_b is determined by ΔP, g_c and A.

Conclusion

Berry transpiration of three grape cultivars was measured using two methods and the results were in good agreement. Variations in VPD, berry size, and cuticular conductance determined daily and developmental changes and cultivar differences in berry transpiration rate. Being predominantly cuticular, berry transpiration was strongly dependent on VPD, which resulted in pronounced diurnal fluctuations. Developmentally, berry transpiration (both per berry and, to a lesser extent, per surface area) peaked when berries were red/purple. On a per berry basis, transpiration correlated strongly with berry surface area from the green hard to blue stages. This relationship did not hold after berries exceeded 20 Brix due to a decline in cuticular conductance. This developmental pattern in berry transpiration was evident in all three cultivars. Although Concord had much lower cuticular conductance than Merlot and Syrah, transpiration per berry was similar among the three cultivars due to the greater size of Concord berries.

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